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# Reply to: "Understanding triphasic HCV decline during treatment in the era of *IL28B* polymorphisms and direct acting antiviral agents via mathematical modeling"

**Triphasic decline in HCV RNA during peginterferon and ribavirin therapy for HCV genotype 1**

*To the Editor:*

We thank Dahari *et al.* for their comments on our recent manuscript investigating the relationship between *IL28B* SNP 12979860 and early HCV RNA kinetics in 173 African American (AA) and 188 Caucasian American (CA) patients infected with chronic HCV genotype 1, during peginterferon alfa-2a and ribavirin (PegIFN-α-2a/RBV) therapy in the VIRAHEP-C study [1]. Compared to SNP C/T and T/T genotypes, the C/C genotype was associated with greater phase 1 and faster phase 2 declines in HCV RNA in both AA and CA patients. Thus, we concluded that this SNP predicts both interferon (IFN) effectiveness at blocking HCV production (i.e., phase 1) and clearance of infected hepatocytes (i.e., phase 2), and that this could explain how the *IL28B* SNP 12979860 C/C genotype increases the probability of SVR (compared to non-C/C genotypes) following PegIFN-α-2a/RBV treatment for HCV genotype 1. Differences in early viral kinetics between AA and CA could be largely explained by racial differences in the frequency of the *IL28B* SNP C allele.

Patients with *IL28B* SNP non-C/C genotypes were more likely to have either a static phase or an increase in serum HCV RNA (i.e., shoulder) from day 2–7. We hypothesized that the *IL28B* SNP-related variability in the shoulder phase was due primarily to differences in IFN effectiveness (SNP C/C > C/T and T/T), as the decline in HCV RNA from day 2–7 was more strongly correlated with the magnitude of phase 1 than the phase 2 slope. In addition, we noted that the rebound in HCV RNA was temporally related to the decline in serum peginterferon from day 2/3 to 7 between the first and second injection [2]. Furthermore, we cited evidence that ribavirin also plays a significant role in regulating viral kinetics between day 2–7 [3]. It is known that ribavirin has no effect on phase 1, either when administered alone or when combined with peginterferon alfa; when combined with peginterferon, it prevents an increase (rebound) in HCV RNA from day 2–7 and accelerates the fall in HCV RNA from day 7–28 (phase 2), in patients with an adequate first phase response [3]. With regard to ribavirin, viral kinetics models that include ribavirin and IFN effectiveness are better able to explain early HCV RNA kinetics than models with IFN effectiveness alone [4]. According to this model, the requirement for ribavirin during phase 2 is inversely related to IFN effectiveness. Thus, phase 2 and SVR in patients with *IL28B* SNP C/C are expected to be less dependent on ribavirin than in patients with non-C/C genotypes.

In their letter, Dahari *et al.* reviewed the prevailing concepts of the shoulder phase from day 2–7 incorporating observations from 2 studies of US and Brazilian patients who were co-infected with HCV and HIV [5,6]. These studies are limited by small sample sizes and inclusion of patients with HCV genotypes 2 and 3. Analogous to our study, patients in one study were stratified by *IL28B* SNP genotype [6]. In keeping with our results, HCV genotype 1, co-infected patients with SNP C/C genotypes had significantly higher first (day 0–2) and second (day 2–29) phase declines in serum HCV RNA [6]. In addition, the peginterferon alfa serum concentration associated with half maximum IFN effectiveness (EC50) was lower in patients with *IL28B* SNP C/C genotypes compared to non-C/C genotypes ($p = 0.02$), suggesting that patients with C/C genotype were more sensitive to peginterferon. Dahari *et al.* suggested that the faster declines in serum HCV RNA from day 2–7 in patients with *IL28B* SNP C/C in our study may be partly related to greater IFN sensitivity (peginterferon EC50) and a higher infected cell loss rate. Our results are consistent with the concept that the shoulder phase reflects both IFN effectiveness and the infected cell loss rate. The peginterferon EC50 (IFN sensitivity) hypothesis is intriguing. However, the difference in peginterferon EC50 between C/C and non-C/C genotypes was not significant in HIV-co-infected patients with HCV genotype 1 ($p = 0.6$), possibly due to inadequate statistical power. Studies with larger numbers of patients infected with HCV genotype 1 alone are needed to address this question and to determine if peginterferon EC50 is an independent predictor of the shoulder and second phase responses.

In another study, Dahari *et al.* observed significantly lower ribavirin plasma concentrations during the first 2 weeks and lower ribavirin exposure or area under the concentration–time curve (AUC) levels at days 3, 7, 14, and 28 of peginterferon alfa combination treatment in HCV-HIV co-infected patients who achieved an SVR relative to those without an SVR [5]. They raised the possibility that lower early ribavirin levels in patients with *IL28B* CC genotypes might explain the transient viral declines and shoulder phase during the first week in our report. In more recent studies, we have investigated the importance of ribavirin pharmacokinetics to the lower efficacy of peginterferon and ribavirin therapy in AA compared to CA with HCV genotype 1 [7,8].

The results suggest that ribavirin drug exposure is a key factor determining HCV RNA dynamics during the shoulder (day 2–7) in patients with HCV genotype 1 regardless of *IL28B* SNP genotype. We studied 75 AA and 75 CA who were more than 90% adherent to PegIFN-α-2a and weight-based ribavirin during the first 24 weeks of therapy in the VIRAHEP-C, based on electronic drug monitoring [7,8]. Compared to CA, AA had lower plasma ribavirin concentrations at weeks 1, 2, and 4, and lower ribavirin exposure (AUC) from week 1–12 that were explained by a 50% higher volume of ribavirin distribution [7,8]. Ribavirin exposure (AUC) during the first week ($AUC_{0-7}$), *IL28B* SNP genotype, age, and platelet count were independent predictors of response (undetected serum HCV RNA) at week 24 (WK24VR) and week 72 (SVR), in a multivariable regression model [8]. Among AA, ribavirin $AUC_{0-7}$ levels were higher in responders at weeks 24 and 72. Ribavirin $AUC_{0-7}$ cut-offs or thresholds that best distinguished WK24VR ($\geqslant$4095 ng/ml d) and SVR ($\geqslant$4480 ng/ml d) from non-responders were identified using receiver operating characteristics curve analysis [8]. Fewer AA (49% and 44%) achieved the respective $AUC_{0-7}$ thresholds than did CA (70% and 64%). In addition, WK24VR and SVR rates were higher in patients with threshold compared to subthreshold ribavirin $AUC_{0-7}$ levels. Yet, there were no differences in WK24VR and SVR between AA and CA with threshold ribavirin $AUC_{0-7}$ levels, providing strong evidence that optimum ribavirin drug exposure during the first week can overcome the racial disparity in WK24VR and SVR.

It is worth noting that WK24VR and SVR to peginterferon and ribavirin did not vary by ribavirin exposure category in patients with the *IL28B* SNP C/C genotype [8]. In contrast, patients with *IL28B* SNP non-C/C and threshold ribavirin $AUC_{0-7}$ levels had significantly higher WK24VR and SVR rates than those with subthreshold levels. The importance of ribavirin exposure on treatment outcomes in this study was reflected in the early HCV RNA kinetics. Compared to CA, AA had a shoulder in serum HCV RNA from day 2–7 and smaller declines in HCV RNA on days 7, 14, and 28 [8]. The shoulder phase was observed in AA with subthreshold, but not in those with threshold ribavirin $AUC_{0-7}$. HCV RNA kinetics from day 0 to 28 did not differ between AA and CA with threshold ribavirin $AUC_{0-7}$. Regardless of *IL28B* SNP genotype, patients with subthreshold ribavirin $AUC_{0-7}$ experienced a shoulder phase from day 2–7. However, phase 2 did not vary by ribavirin $AUC_{0-7}$ category in patients with *IL28B* SNP C/C. In contrast, declines in HCV RNA from day 2–7 and day 7–28 (phase 2) were greater in non-C/C patients with threshold compared to subthreshold ribavirin $AUC_{0-7}$ levels. Serum peginterferon and ribavirin levels did not vary by *IL28B* SNP genotype. These results indicate that suboptimum ribavirin exposure was responsible for the shoulder in HCV RNA from day 2–7 during peginterferon combination treatment, and are consistent with the concept that due to higher IFN effectiveness and infected cell loss rates, the phase 2 (day 7–28) decline rate is less dependent on threshold ribavirin exposure in patients with HCV genotype 1 who carry the *IL28B* SNP C/C genotype.

## Conflict of interest

C.H. serves on the Roche-Genentech Pegasys Advisory Board.

## References

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